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Lesch-Nyhan Syndrome: Altered Kinetic Properties of Mutant Enzyme

Abstract. *Hypoxanthine-guanine phosphoribosyltransferase is virtually inactive in erythrocytes from patients with the classical Lesch-Nyhan syndrome. In one such patient, activity of this enzyme ranged from 8 to 34 percent of normal in erythrocytes when assayed with a very high concentration of magnesium 5-phosphoribosyl-1-pyrophosphate. In addition, the mutant enzyme exhibited sigmoidal kinetics with this substrate as well as an increased Michaelis constant for both guanine and hypoxanthine. These findings provide the first evidence for genetic heterogeneity within the group of patients with the Lesch-Nyhan syndrome.*

Hypoxanthine-guanine phosphoribosyltransferase (PRT) (E.C. 2.4.2.8) catalyzes the transfer of the 5-phosphoribosyl moiety of magnesium 5-phosphoribosyl-1-pyrophosphate (MgPRPP) to guanine and hypoxanthine to form their corresponding ribonucleotide derivatives, guanosine 5′-monophosphate (GMP) and inosine 5′-monophosphate (IMP). A genetic deficiency of this X-linked enzyme is associated with two clinical syndromes in man. A partial deficiency (with values up to 17 percent of normal in erythrocytes) leads to excessive uric acid production and the consequent development of uric acid calculi, hyperuricemia, and gouty arthritis; of these patients 20 percent exhibit mild neurologic symptoms (*1*). In contrast, the virtually complete deficiency of this enzyme in man (< 0.5 percent of normal activity) is associated with the Lesch-Nyhan syndrome, a bizarre behavioral disorder characterized by self-mutilation, choreoathetosis, spasticity, and mental retardation as well as increased uric acid production (*2*).

Activity of PRT in lysates of erythrocytes from a 10-year-old male with all of the classical features of the Lesch-Nyhan syndrome was approximately 0.2 percent of normal when assayed at a concentration of MgPRPP ($1 \times 10^{-3}M$) which is saturating for the normal enzyme. However, at a higher concentration of MgPRPP ($1 \times 10^{-2}M$), activity of this mutant enzyme ranged from 6 to 8 percent of normal with guanine as substrate to 34 percent of normal with hypoxanthine as substrate (Table 1). Extrapolating to saturating concentrations of these purine substrates, we found that the activity of the mutant enzyme was 12 percent of normal with guanine and 94 percent of normal with hypoxanthine. These values are within or above the range of PRT activity observed in gouty subjects with no neurological dysfunction. Hemolyzates from five other patients with the Lesch-Nyhan syndrome had no detectable activity (< 0.01 percent of normal) at MgPRPP concentrations as high as $1 \times 10^{-2}M$.

This mutant enzyme exhibited qualitatively as well as quantitatively altered kinetics with MgPRPP as the variable substrate. The effect of increasing MgPRPP concentration on rate of GMP production by normal and mutant PRT enzymes is shown in Fig. 1. The MgPRPP substrate curve observed with the mutant enzyme appeared to exhibit sigmoidal characteristics in contrast to the hyperbolic kinetics observed with the normal enzyme (*3*, *4*). The normal PRT enzyme has been

Table 1. Comparison of the kinetic properties of the normal and mutant hypoxanthine-guanine phosphoribosyltransferase (PRT) enzymes. Erythrocyte PRT enzyme activity was measured (*8*) with $1 \times 10^{-3}M$ MgPRPP (for the normal enzyme) or $1 \times 10^{-2}M$ MgPRPP (for the mutant enzyme) and either $1 \times 10^{-4}M$ [8-^{14}C]guanine (1.76×10^4 dpm/nmole, 1.15×10^5 dpm/nmole) or $1 \times 10^{-4}M$ [8-^{14}C]hypoxanthine (9.28×10^3 dpm/nmole) as purine substrates. The PRT activity in extracts of cultured skin fibroblasts (*9*) was measured similarly, except that 3.3 m*M* thymidine triphosphate was included to inhibit 5′-nucleotidase activity (*5*). The K_m of the normal enzyme for both MgPRPP and the purine substrates was estimated from a curve of $1/v$ plotted against $1/S$. The K_m of the mutant enzyme for guanine and hypoxanthine was also estimated from double reciprocal plots, while the $S_{0.5}$ for MgPRPP (that is, the MgPRPP concentration giving one-half of the maximum observed rate) was estimated from curves of v plotted against MgPRPP concentration. Product formation was proportional to enzyme concentration and time under the conditions of the experiments shown for both the normal and mutant enzymes.

Enzyme source		Phosphoribosyltransferase activity (nmole mg^{-1} hr^{-1})		Apparent $K_{m(MgPRPP)}$ (M)		Apparent $K_{m(guanine)}$ (M)	Apparent $K_{m(hypoxanthine)}$ (M)
Cell	Type	Guanine	Hypoxanthine	Guanine	Hypoxanthine		
Erythrocyte	Normal	98 ± 14*	97 ± 19*	2.5×10^{-4}		5.0×10^{-6}	1.7×10^{-5}
	Mutant	8.2 (12.1)†	33.7 (94.4)†	3.2×10^{-3}‡	2.8×10^{-3}‡	4.8×10^{-5}	1.8×10^{-4}
Fibroblast	Normal	141 ± 17§		1.0×10^{-4}			
	Mutant	10.4		$> 2.0 \times 10^{-3}$‡			

* Mean ± S.D. in 119 subjects. † Numbers in parentheses represent V_{max} of the mutant enzyme for each purine substrate calculated from the Michaelis-Menten formula ($V_{max} = v(K_m + S)/S$). § Mean ± S.D. in 13 normal cell strains. ‡ Values depict $S_{0.5}$ rather than K_m.

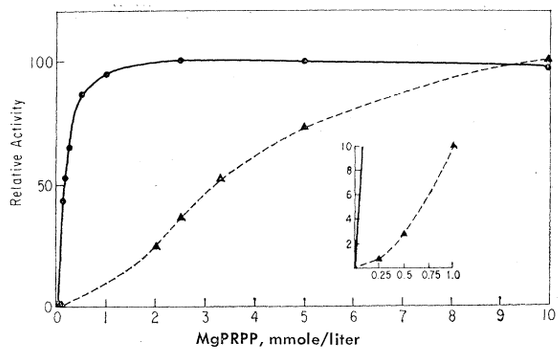

Fig. 1. Effect of MgPRPP concentration on GMP synthesis by normal and mutant PRT enzymes. PRT activity was assayed as described in the legend to Table 1 with $1 \times 10^{-4}M$ guanine as nonvariable substrate and the indicated concentration of MgPRPP. The data are expressed as relative activity ($v/V \times 100$, where v is the observed velocity and V is the approximate maximum velocity), using the GMP formed at $2.5 \times 10^{-3}M$ and $1 \times 10^{-2}M$ MgPRPP as V for the normal (70.2 nmole per milligram of protein per hour) and mutant (6.1 nmole per milligram of protein per hour) enzyme, respectively. Virtually identical results were obtained with hypoxanthine as the fixed substrate. Solid line, normal enzyme; broken line, mutant enzyme.

shown to exhibit sigmoidal kinetics at low ratios of Mg^{2+} to PRPP (*4*). However, sigmoidal kinetics were also obtained when the mutant enzyme was assayed at a constant ratio of 2 Mg^{2+} to MgPRPP or at $5 \times 10^{-2}M$ Mg^{2+} (data not shown), suggesting that the sigmoidicity observed was not a reflection of insufficient Mg^{2+}. Hill plots of the MgPRPP substrate curves for the normal and mutant enzyme were linear and gave n values (slope) of 1.0 and 2.5 (range 2.3 to 2.8), respectively.

The apparent K_m (Michaelis constant) of the normal enzyme for MgPRPP was $2.5 \times 10^{-4}M$, which agrees well with reported values (*3, 4*). However, the MgPRPP concentration required for one-half of the maximum observed rate ($S_{0.5}$) of the mutant enzyme ranged from 2.8 to $3.2 \times 10^{-3}M$, or 13 times higher than the apparent K_m of the normal enzyme for MgPRPP. As illustrated in Fig. 2, $10^{-2}M$ MgPRPP failed to confer stability to the mutant enzyme during thermal inactivation at 80°C, whereas the normal erythrocyte PRT enzyme was partially protected by MgPRPP at concentrations as low as $5 \times 10^{-5}M$ under the same conditions. The failure of high MgPRPP concentrations to protect the mutant enzyme indicates that either MgPRPP was not significantly bound under the experimental conditions (high temperature, no purine substrate) or that binding of MgPRPP did not influence the rate of heat inactivation. Thus, the results of the heat inactivation study do not provide direct confirmation of altered MgPRPP binding by the mutant enzyme, but clearly indicate a major physical difference between the mutant and normal enzymes independent of our kinetic studies.

The PRT enzyme in extracts of cultured fibroblasts derived from this individual also exhibited apparent sigmoidal kinetics and an increased $S_{0.5}$ with MgPRPP as the variable substrate. This indicates that the altered kinetic properties observed with the mutant enzyme were not limited to one cell type. However, definitive kinetic studies of the mutant enzyme in crude fibroblast extracts were impossible because of the high levels of 5′-nucleotidase activity in this tissue even in the presence of thymidine 5′-triphosphate, a competitive inhibitor of 5′-nucleotidase (*5*).

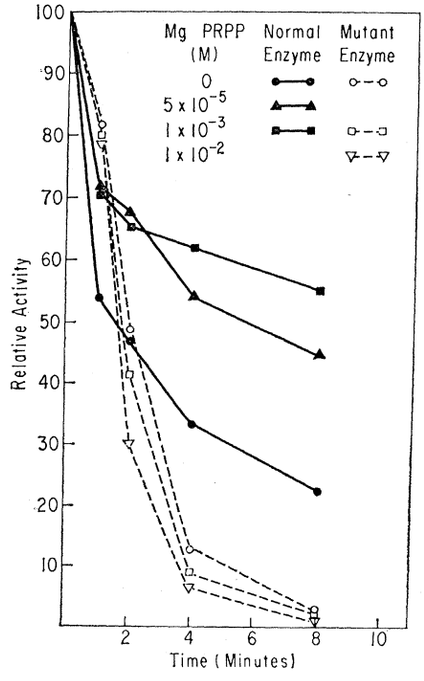

Fig. 2. Effect of MgPRPP on rate of inactivation of normal and mutant erythrocyte PRT enzymes at 80°C. The normal and mutant enzyme preparations (containing 10 to 30 mg protein) were incubated at 80°C in a 1.0-ml reaction mixture containing 0.01M tris HCl, pH 7.4, and the indicated amount of MgPRPP. Duplicate 0.05-ml portions were withdrawn at the specified times, chilled rapidly, and assayed for PRT activity (Table 1). The initial activity at $t = 0$ has been taken as V, and residual PRT activity is expressed as percent of this control value ($v/V \times 100$).

Both the normal and mutant erythrocyte PRT enzymes exhibited Michaelis-Menten kinetics with guanine or hypoxanthine as the variable substrate. However, the apparent K_m of the mutant enzyme for these purine bases was substantially higher than that observed with the normal enzyme (Table 1).

The finding of a PRT enzyme with altered kinetic properties in an individual with the Lesch-Nyhan syndrome is important for several reasons. First, the detection of a human genetic disorder associated with the production of an enzyme with altered kinetic constants appears to be relatively unusual (*6*). Second, this variant provides the first direct evidence for genetic heterogeneity within the group of patients with the classical Lesch-Nyhan syndrome and indicates that the enzyme defect, at least in this individual, results from an alteration in the structural gene coding for this enzyme. Third, the sigmoidicity of the MgPRPP rate curve observed with the mutant enzyme may reflect cooperative interaction of a substrate with an enzyme or allosterism (*7*). This alteration in the kinetic behavior of the mutant enzyme presumably resulted from a single mutation in a structural gene. While in this case the end result is deleterious, the reverse could be true, and selection pressure for the altered protein could exist. Finally, therapy designed to increase the intracellular concentration of MgPRPP or the purine bases guanine and hypoxanthine may be beneficial to this patient.

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Receptive Field Mechanism in the Vertebrate Retina

Abstract. *In the catfish retina there are two types of ganglion cells: in one type (type A cell) a spot of light at the center of its receptive field gives rise to a sustained discharge whereas an annulus gives rise to a transient response, and in the other type (type B cell) the response pattern is reversed for a spot and an annulus. Current injected into the horizontal cell induces spike discharges of the ganglion cell very similar to that elicited by a spot of light or by an annulus. In both types of receptive fields, depolarization of the horizontal cell gives rise to a response of the ganglion cell similar to that elicited by a spot of light, whereas hyperpolarization of the cell gives rise to a response of the ganglion cell similar to that elicited by an annulus. Current through a single injecting electrode could drive two types of cells simultaneously. Interaction between a spot of light and an annulus can also be simulated by replacing one light stimulus by current of the proper polarization injected into the horizontal cells. Results suggest that interactions among three neuronal structures, the receptor, the horizontal cell, and the bipolar cell, produce the basic receptive field organization in the channel catfish.*

The organization of the receptive field of the vertebrate retinal ganglion cell has been a subject of intensive study (*1, 2*). Although the receptive field has been shown to consist of several subfields (*3*), the possible mechanisms underlying the organization have not often been explored. An exception could be found in the mudpuppy (*4*). In this report I present functional evidence which suggests that interaction among three neuronal structures, the receptor, the horizontal cell, and the bipolar cell, produces the basic structure of the receptive field.

The catfish retina possesses distinct advantages for the study of the organization of the receptive field because (i) the organization of the receptive field is simple (*5*), (ii) the horizontal cells are always hyperpolarized by light (*6*), and (iii) the spike discharge can be, as will be shown here, initiated by injecting current into the horizontal cells (*7*).

The excised eye of the channel catfish (*Ictalurus punctatus*) was used throughout the experiment, and the preparation was kept moderately dark-adapted. Under such conditions both the response from the horizontal cell and the spike discharges from the ganglion cell (*8*) were generated by signals from a single class of cones with a maximum sensitivity at 625 nm (*5, 6*). This provision excluded a possible complication by color-coded mechanisms such as that reported for the goldfish retina (*2, 3*). The response of the horizontal cell was recorded by means of a glass pipette filled with potassium citrate (resistance, 100 to 200 megohms) and the spike discharge was recorded by the use of one or two tungsten electrodes. The level of the intracellular potential of the horizontal cell was artificially altered by injecting current through the recording electrode, and the amount of current passed was monitored by a voltage created across a 1-kohm resistor placed between the preparation and the ground. As shown in Figs. 1 and 2, the rise and decay time of the current were adjusted so that they approximated the rise and decay time of the response of the horizontal cell. In the experiments for which results are shown in Figs. 1 and 2 the tips of both recording electrodes were placed within the retinal area illuminated by the central spot, and the two electrodes were approximately 0.2 mm apart. The magnitude of the current injected into the horizontal cell to initiate the spike discharge of the ganglion cell 0.2 mm away was less than 20 na. A two-channel photostimulator provided a central spot of 0.3 mm and an annulus with an inner diameter of 0.35 mm. The outer diameter of the annulus was 5.0 mm, which was roughly two-thirds of the diameter of the dissected eyecup preparation. The two stimuli were monochromatic lights of 525 nm, and they were placed concentrically on the retina. The intensity of flash used was 1.0 to 1.8 logarithmic units below $I_{1/2}$, the flash intensity required to give the horizontal cell response of half the maximal amplitude [see also equation 1 in (*6*)].

In the catfish, polarization of the horizontal cell potential by means of current passed through the recording electrode produced discharges of the ganglion cell very similar to that caused by the light stimulus. The possibility that this discharge might have been due to the direct effects of current on the ganglion cell was excluded because the injected current could initiate the ganglion cell discharge only when the electrode was at a position where it could record the response of the horizontal cell, and dislocation of the electrode resulted in the inability of the injected current to activate the spike discharge.

Furthermore, current injected through an electrode placed 1.0 to 1.5 mm away from the spike recording site was still effective (at a current intensity of 30 to 50 na) in inducing the discharge of the ganglion cell. Current injected through a single electrode was also effective in inducing the spike discharge of two types of ganglion cells (types A and B) recorded simultaneously by two tungsten electrodes. These observations indicate that the current injected into a horizontal cell could spread laterally as in the case of the potential change induced by photic